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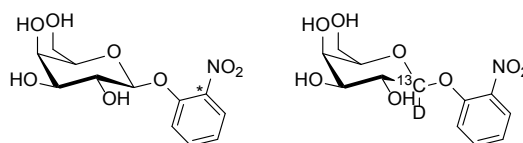
## DNP NMR of carbohydrate converting enzymes

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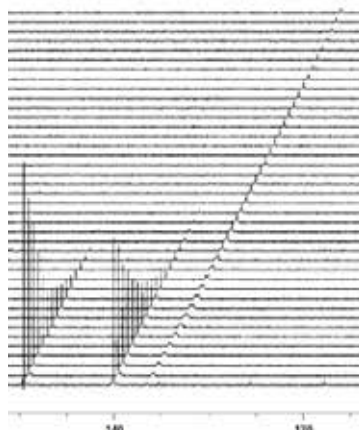
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Dissolution dynamic nuclear polarization (DNP) NMR can be used to increase the sensitivity of  $^{13}\text{C}$  NMR signal by up to four orders of magnitude [1]. This allows for real time monitoring of reactions and observation of intermediates [2]. The biggest drawback of the method is the loss of polarization with  $T_1$  relaxation, but even with this limitation, it is possible to obtain detailed reaction parameters in less than one minute. The enzyme investigated was  $\beta$ -galactosidase from *E. coli* (E.C. 3.2.1.23). It is well described and the mechanism is generally accepted to be a double displacement with a covalently bound intermediate, however, this evidence is based on mutant of X-ray crystallography and simulations [3]. As the natural substrate lactose does not have any quaternary carbon with long  $T_1$ , the unnatural substrate *o*-nitrophenyl  $\beta$ -D-galactopyranoside was used (figure 1) as the quaternary positions have  $T_1$  relaxations of ca. 15 s instead of <2 s.



**Figure 1.** Left: *o*-nitrophenyl  $\beta$ -D-galactopyranoside. The marked (\*) position is the one monitored in figure 2. Right: Doubly isotopically labelled substrate on the 1-position which increases the  $T_1$  from <2 s to ca. 10 s.

The DNP NMR monitoring of the hydrolysis of this substrate can be seen in figure 2, and another use of this substrate is for optimizing the conditions for a labelled substrate (figure 1), which would further increase the signal and allow monitoring of the carbohydrate instead of the aglycon. This is, however, not commercially available and had to be synthesized from doubly labelled galactose.



**Figure 2.** DNP NMR of the enzymatic hydrolysis of *o*-nitrophenyl  $\beta$ -D-galactopyranoside. There is 2 seconds between each  $10^\circ$  flip angle scan and each new spectrum is shifted 0.5 ppm.

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[3] Juers, D. H.; Matthews, B. W.; Huber, R. E. LacZ  $\beta$ -Galactosidase: Structure and Function of an Enzyme of Historical and Molecular Biological Importance. *Protein Sci.* **2012**, *21*, 1792–1807.